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Title:

Green extraction of jack bean protein using aqueous-deep eutectic solvent system: Impact on protein structure, functionality, and emulsifying properties

Authors & affiliations:

Feyisola Fisayo Ajayi^a, Akmal Nazir^a, Priti Mudgil^a and **Sajid Maqsood^{a*}**

^a Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, 15551, United Arab Emirates.

Email: sajid.m@uaeu.ac.ae

Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text into it.)

Preparation of Your Abstract

1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word **ONLY** (place names excluded). No full stop at the end.

2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.

Introduction: Clearly state the purpose of the abstract

Methods: Describe your selection of observations or experimental subjects clearly

Results: Present your results in a logical sequence

Discussion: Emphasize new and important aspects of the study and conclusions that are drawn from them

The demand for sustainable protein extraction methods is rising due to the limitations of conventional solvents. Deep eutectic solvents (DES) provide an eco-friendly alternative, offering enhanced efficiency while reducing environmental impact. This study formulated four choline chloride-based aqueous DES (ADES) systems with different hydrogen bond donors (glycerol, xylitol, malic acid, and urea) to extract jack bean proteins (JPs). Extracted proteins were evaluated against alkaline-extracted JPs in terms of structural, functional, and oil-in-water emulsification. FTIR confirmed variations in intermolecular interactions among ADES systems. ADES-xylitol achieved the highest protein recovery (76.39%), while alkaline extraction yielded slightly higher protein content (81.79%) but was not significantly different ($p > 0.05$) from ADES-urea (81.17%). SDS-PAGE showed that ADES systems extracted all protein subunits, unlike alkaline extraction, which lacked the 35 kDa legumin acidic subunit. Alkaline extraction resulted in superior solubility (61.04%) and emulsifying activity (39.53 m²/g), while ADES-urea had the best foaming properties (activity: $114.81 \pm 5.23\%$, stability: $92.59 \pm 0.47\%$). Regarding oil-in-water emulsification, alkaline and ADES-glycerol extracted proteins produced small droplet sizes ($D_{4,3}$: $1.58 \pm 0.00 \mu\text{m}$ and $1.62 \pm 0.02 \mu\text{m}$, respectively) with good creaming stability after seven days.. These results highlight ADES-glycerol as a promising green solvent for JP extraction, with potential application as a sustainable food emulsifier.